during slipknotting. Secondly, show that SBM can be used as a tool to guide experiments to untie a knot and to explore the function of protein knots.

2389-Pos Board B81

The Molten Globule State of Maltose Binding Protein: DEER Measurements at PH 3

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Maltose-binding protein (MBP) is in a molten globule state at pH 3 as characterized by ANS binding. DEER measurements of seven spin-labeled double mutants as in the native state at pH 7 had shown excellent agreement with X-ray data. At pH 3 corresponding DEER measurements of all the mutants yield a broad distribution of distances. This was to be expected if there is no defined tertiary structure and the individual helices pointing into all possible directions. However, as MBP still binds maltose as molten globule although more weakly, the native structure must be retained at or near the active site. This is now being investigated with a new set of mutants.

2390-Pos Board B82

Conformational Dynamics of a Fast Folding Cytochrome Captured by Time-Resolved Spectroscopy

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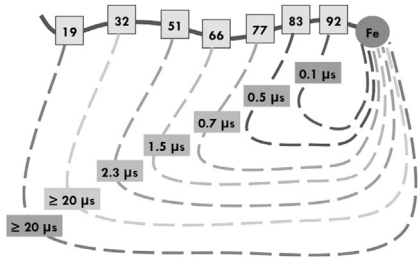
We probe intrachain contact dynamics in unfolded cytochrome cb562 by monitoring heme quenching of excited ruthenium photosensitizers covalently bound to residues along the polypeptide. Tertiary contact formation kinetics provide insight into the upper "speed limit" for protein folding rates. The rate constants exhibit a power-law dependence on the number of peptide bonds between the heme and Ru complex. Adherence of our data to a slope of −1.5 is consistent with theoretical models for ideal, freely-jointed Gaussian chain polymers, but its magnitude is smaller than reported for synthetic polypeptides. We also examine rates of contact formation within a stable loop in a His63-heme ligated form of the protein. Additionally, we resolve millisecond-timescale folding by coupling time-resolved fluorescence energy transfer (trFRET) to a continuous flow microfluidic mixer to obtain intramolecular distance distributions throughout the folding process. Our results suggest that cytochrome cb562 is minimally frustrated.



2391-Pos Board B83

Roles of Hydrophobic Interactions and Hydrogen Bonds in Beta-Sheet Formation

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In this presentation, we will discuss interactions of extended conformations of homodimeric peptides made of small (glycine or alanine) and large hydrophobic (valine or leucine) sidechains using all-atom computer simulations to decipher driving forces behind beta-sheet formation. Dimers adopt beta-sheet conformations at short inter-peptide distances (x ~ 0.5 nm) while at intermediate distances (~ 0.8 nm), dimers made of valine or leucine assume ''cross-beta like'' conformations with sidechains interpenetrating each other. These two states are identified as minima in the Potential of Mean Force (PMF). While the number of inter-peptide hydrogen bonds increases with decreasing inter-peptide distance, the total hydrogen bond number in the system does not change significantly, suggesting that formation of beta-sheet structures from extended conformations is not driven by hydrogen bonds. This is confirmed by an increase in electrostatic energy at short inter-peptide distances. A remarkable correlation between the volume of the system and the total electrostatic energy is observed, supporting the view that excluded water regions in proteins have an enthalpic penalty. We will also discuss microscopic mechanisms accounting for beta-sheet formation based on computed enthalpy and entropy and we will show that they are different for peptides with small and large sidechains.

2392-Pos Board B84

The Analysis of the Conservation of Folding Cores Among Highly Diverse Proteins in the Lysozyme Superfamily

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For understanding a protein folding mechanism it is important to know whether folding mechanisms of evolutionary related proteins are conserved during biological evolution. However, the relationships between evolution of proteins and protein folding mechanisms are not completely elucidated. How the information of folding mechanisms reflects on amino acid sequences is an unsolved problem. The trace of this information of an ancestral protein during sequence divergence should be found on a sequence. In the lysozyme superfamily the structural similarity suggests they have a common ancestor despite their low sequence similarity. In this study, we analyze the folding mechanism of each protein in the lysozyme superfamily only from their amino acid sequences by means of the inter-residue average distance statistics. In these analyses we predict possible folding initiation sites as predicted folding core by analyzing contact maps based on the statistics. If the distributions of predicted folding cores among homologous proteins are similar, homologous proteins have robust folding cores not altered by amino acid substitutions. Multiple sequence alignment is used to check the existence of robust folding cores in each family of lysozyme whose sequences are not so diverged. Structural alignment is also used to investigate the correspondence of robust folding cores to common structural units among highly diverged sequences in the lysozyme superfamily. From the comparison of robust folding cores corresponding to structurally similar regions, we examine the existence of robust folding cores among distantly related proteins. The results suggest that predicted robust folding cores in closely related sequences of each family of lysozyme tend to correspond to common structural units. Thus it is indicated the existence of robust folding cores among the lysozyme superfamily although they lost sequence homology.

2393-Pos Board B85

Coiled-Coil Probes Capture Mechanical Unfolding Pathways of Individual Proteins

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Force-induced unfolding of a consensus ankyrin repeat protein NI_6C was suggested to follow a vectorial pathway from the C to the N terminus. We further test this vectorial unfolding hypothesis by introducing, at the gene level, a 70 amino acid coiled-coil (CC) polypeptide probe at different positions along the $NI_{10}C$ sequence and determine the unfolding force extension data of the hybrid proteins with single-molecule atomic force spectroscopy (AFM). The basic idea assumes that the coiled-coil folds independently of the host protein and does not significantly disturb its structure, and that the occurrence and the position of the CC unfolding force peak is indicative of the progress of the unfolding process past the location of the CC probe. First, AFM measurements of the CC probe flanked by I27 domains captured a single ~35pN unfolding peak characteristic of the unzipping of the coiled-coil. Second, the unfolding force-extension data of the NI_8CCI_2C hybrid indicated that the unfolding of the CC occurs early in the unfolding process, consistent with the unfolding starting at the C terminus. Third, the force-extension data of the NI_4CCI_6C indicate that the unfolding of the CC occurs late during the unfolding process, corroborating our vectorial unfolding hypothesis. More generally, our studies suggest that coiled-coil probes maybe very useful to examine mechanical unfolding pathways of many proteins, including large proteins that are rarely studied in bulk folding measurements.

Protein-Ligand Interactions and Enzymes

2394-Pos Board B86

Isomerization and Autolysis at Specific Amino Acid Residues of the Tau Protein and its Relations to Alzheimer's Disease

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Alzheimer's Disease is the most common form of dementia that more than 5 million Americans are suffering from. With no known cure and no known cause, it is an urgent matter that researcher's are desperately attempting to tackle. Alzheimer's disease is clinically characterized by memory loss and histopathologically characterized by the high prevalence of amyloid plaques and neurofibrillary tangles (tau tangles). In a healthy brain, the tau protein performs the role of stabilizing microtubules in healthy neurons. However, due to their long turn-over rates, they are susceptible to many